Inhibition of smooth muscle cell migration and neointima formation in vein grafts by overexpression of matrix metalloproteinase-3

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Objective: **Saphenous vein grafts suffer from neointima formation following bypass surgery. Matrix metalloproteinases (MMPs) play important roles in this process. We examined MMP-3 for its therapeutic potential to prevent smooth muscle cell migration and neointima formation in venous bypass grafts using adenovirus-mediated gene transfer.**

Methods: **Human aortic smooth muscle cells (HASMC) were transduced with adenoviral vectors encoding ß-galactosidase (AVEßgal) or human MMP-3 (hMMP-3), and characterized for migration in the amniotic membrane stroma as an in vitro model of the vascular wall. Cholesterol-fed New Zealand white rabbits underwent jugular vein bypass grafting into carotid arteries. Before insertion, grafts were incubated ex vivo with either AVEßgal or hMMP-3. Transgene expression was characterized by immunohistochemistry and in situ zymography. Grafts (n = 6) were explanted after 28 days and intimal hyperplasia was quantified.**

Results: **Migration of HASMC was significantly reduced when transduced with hMMP-3 compared to controls ($P < .001$). Immunocytochemistry of hMMP-3 transduced venous grafts localized this protein to the intima. In situ-zymography showed increased MMP activity in the intima of hMMP-3 transfected grafts. Stenosis degree ($P = .001$), intima/media-ratio ($P = .023$) and lesion thickness ($P = .003$) were significantly reduced in grafts transduced with Ad.MMP-3 in comparison to controls. There was no difference inside control groups.**

Conclusion: **MMP-3 overexpression inhibits formation of intimal hyperplasia in arterialized vein grafts. Adenovirus mediated gene transfer of MMP-3 may be of clinical use to prevent vein graft stenosis following bypass surgery. (J Vasc Surg 2009;49:750-8.)**

Clinical Relevance: **Although vein grafts are conduits of choice for lower extremity and coronary bypass graft surgery, patency rates of vein grafts remain unsatisfactory. The underlying mechanism for graft stenosis and occlusion is the development of intimal hyperplasia, caused by smooth muscle cell migration from the vascular media into the intima. The inhibition of smooth muscle cell migration by gentherapeutic strategies, clinically applicable to veins after harvesting and before graft insertion, may lead to bypass grafts with improved patency. Here we examine the effect of overexpression of MMP-3 both in vitro on smooth muscle cell migration and in vivo on development of intimal hyperplasia, aimed to develop pre-clinical strategies for prevention of vein graft stenosis.**

Autologous vein grafts are the conduits of choice for lower extremity and coronary artery bypass graft surgery. However, only 50%-80% of vein grafts remain patent at 5 to 15 years.[1-5] Intimal hyperplasia is the major cause of vein graft stenosis. Smooth muscle cell (SMC) proliferation and migration into the intima and their proliferation are key mechanisms in this process.[6,7] Cell migration requires extracellular matrix (ECM) degradation by a variety of extracellular proteinase, among which the matrix metalloproteinases (MMPs) play an important role. MMPs can collectively degrade virtually all ECM components and have been implicated in a variety of tissue remodeling processes, including atherosclerosis and intimal thickening.[8] MMP-3 (stromelysin-1) has the broadest substrate specificity of all MMPs; it degrades collagen types III, IV, and V, laminin, fibronectin, elastin, and proteoglycans. MMP-3 is secreted by a variety of cell types including SMC.[9,10] High levels of MMP-3 have been described in atherosclerotic plaques[11] and in the SMC of atherosclerotic coronary arteries.[10] MMP-3 is therefore considered as a protease supporting SMC-migration and development of intimal hyperplasia in vascular remodeling.

Paradoxically, a common mutation of the MMP-3-promoter resulting in reduced MMP-3 expression is associated with development of atherosclerosis[12] and restenosis.[13] In MMP-3 knock-out mice, atherosclerotic plaques are more instable than in MMP-3 expressing mice.[14] Conversely, high levels of MMP-3 are associated with increased risk of myocardial infarction.[15] Therefore, the role of MMP-3 in vascular remodeling remains unclear.

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Based on the previous findings, we hypothesized that high levels of MMP-3 inhibit vein graft stenosis. Here we report that adenovirus-mediated overexpression of MMP-3 blocks SMC cell migration in vitro and reduces the formation of intimal hyperplasia in arterialized vein grafts.

MATERIALS AND METHODS

Cells and culture medium. Human aortic smooth muscle cells (HASMC) were purchased from Clonetics (San Diego, Calif) and grown in smooth muscle basal medium supplemented with 10 ng/mL of human epidermal growth factor (hEGF), 2 ng/mL of human fibroblast growth factor (hFGF), 0.39 μg/mL of Dexamethasone, 50 μg/mL of Gentamycin, 50 ng/mL of Amphotericin-B, and 5% fetal bovine serum. The cells were used between passage 2 and 8 in culture. Human 293 embryonic kidney cells stably transfected with the E1A and E1B genes (kindly provided by Dr R. Schneider, NYU Medical Center) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

Generation of recombinant adenoviruses and transduction conditions. Adenoviral vectors encoding either ß-galactosidase or human MMP-3 (hMMP-3) were generated as described.[16,17] Briefly, full length DNA for human MMP-3 (or ß-galactosidase) was subcloned into a pCMVAd. The resulting construct, pCMVAd.MMP-3, contained the MMP-3 cDNA flanked on its 5′ end by the cytomegalovirus (CMV) promoter and on its 3′ end by a polyadenylation sequence and several hundred nucleotides of adenovirus type 5 (Ad dL 309) genome lacking the E1A region. Linearized plasmids and the Ad dL 309 viral genome were cotransfected into 293 cells or, for animal experiments, in N52.E6 cells.

HASMC were transduced with 2×10^8 pfu/mL (100 pfu/cell). Transduction efficiency ranged 24%-40% as assessed by ß-galactosidase staining of cells transduced with Ad.ßgal.

Smooth muscle cell invasion of the amniotic membrane. Human amniotic membranes were prepared as described.[16]

Northern blotting. Northern blotting was performed as described[18] using a DIG-labeled cDNA probe to MMP-3 kindly provided by Dr Markku Kurkinen (Wayne State University), or an 18S DIG-labeled rRNA probe (Boehringer, Mannheim, Germany) as a control.

Western blotting and casein zymography. Western blotting and casein zymography were performed as described.[19]

Ex vivo transduction of vein explants with adenoviral vectors. For animal experiments, N52.E6 cell line (human amniocytes, a generous gift from G. Schiedner and S. Kochanek, University of Cologne, Germany) was used for homologous recombination of adenoviral vectors to avoid the generation of replication-competent adenoviral vectors. Before grafting into the carotid arteries, excised jugular veins were incubated for 30 minutes at 37°C under sterile conditions with adenoviral vectors encoding hMMP-3 or ß-galactosidase (2×10^9 pfu/mL of Poloxamer 407). Poloxamer 407, reported to increase virus-mediated transduction efficiency 100-fold,[20] was used to reduce adenovirus concentration and minimize viral toxicity. After incubation, the transduced veins were washed twice with phosphate buffer solution (PBS) to remove residual adenoviral vectors.

Vein graft model of intimal hyperplasia. Female New Zealand white rabbits (Charles River GmbH, Sulzfeld, Germany) of approximately 4 kg were fed a 1% cholesterol diet from day 7 before operation until euthanasia. The rabbits were pre-anesthetized with ketamine (25 mg/kg) and midazolam (0.1 mg/kg) intramuscularly (i.m.). An intravenous (i.v.)-line was placed, 0.04 mg/kg buprenorphine injected and propofol-1% was triggered i.v. for intubation. After intubation, anesthesia was maintained by spontaneous breathing of 40% oxygen and 2%-volume isoflurane. Deepness of narcosis and life parameters were monitored continuously. Standard vein graft dissection techniques were used to collect the left jugular vein, which was transferred to a laboratory designed for work with genetically hazardous material (level SII). Under sterile conditions, the explanted veins were flushed with PBS, Poloxamer 407 or transduced with adenoviral vectors encoding for ß-galactosidase or hMMP-3. After injection of 100 i.u/kg heparin, the veins were grafted end-to-side into the carotid artery with prolene 10-0 (Ethicon, Hamburg, Germany). The animals were extubated and monitored in a recovery room for 6 hours before being transferred back to the animal housing facility. Perioperatively the animals received a single dose of penicillin/streptomycin (0.5 mL tardomycel subcutaneously [s.c.], Bayer AG, Leverkusen, Germany), and three daily s.c. injections of carprofene (25 mg). For harvesting the grafts, the animals were anesthetized with ketamine (25 mg/kg) and midazolam (0.1 mg/kg) i.m., and sacrificed with an i.v. overdose of pentobarbital (300 mg/kg) before making the skin incision. Harvested grafts were washed with PBS and either embedded in OCT and snap-frozen in liquid nitrogen, or fixed for later paraffin embedding. Triplicate animals were used for analyzing hMMP-3 overexpression at postoperative day 7, and 6 animals/group for characterizing intimal hyperplasia. Two animals in our series had occluded grafts due to technical errors and were excluded from analysis.

The animals were treated in accordance with the German animal welfare law and European Union guidelines. Institutional approval for animal experiments was obtained (No. 509.6-42502-99/201). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Immunohistochemistry. Ten-micrometer frozen sections were incubated with mouse anti-MMP3 monoclonal antibody (No. 1339, Chemicon) diluted 1:100 overnight at 4°C, followed by 30 minutes of incubation with FITC-labeled goat-anti-mouse antibody [No. AP 124 F Chemicon], and fixation with mowiol (Hoechst, Frankfurt, Germany). These MMP-3 antibodies do not crossreact with rabbit-MMP-3.

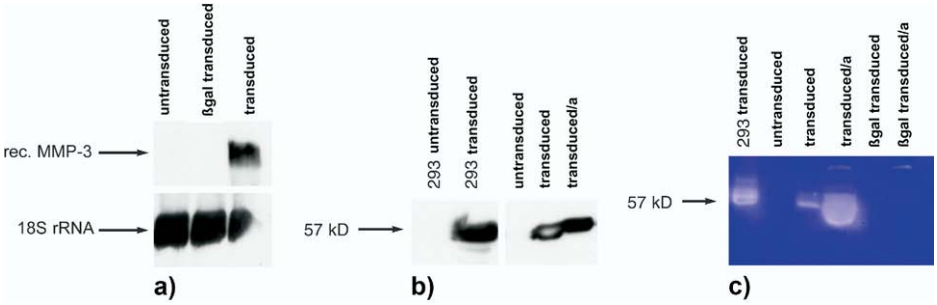

Fig 1. Characterization of hMMP-3 overexpression in HASMC transduced with hMMP-3. **a,** Northern blotting analysis of total ribonucleic acid (RNA) from non-transduced, ß-galactosidase (Ad.ßgal)- or human matrix metalloproteinases (hMMP)-3-transduced human aortic smooth muscle cells (HASMC). Only cells transduced with hMMP-3 express the 2.2 Kb MMP-3 mRNA (rec. hMMP-3). The 18 s rRNA is shown as a control. **b,** Western blotting. Expression of an immunoreactive 57 kDa band consistent with the M_r of pro-MMP-3 is present in hMMP-3-transduced HASMC or 293 cells. HASMC grown on the amniotic membrane (transfected/a) or in a culture dish (transfected) produced comparable levels of MMP-3. **c,** Casein zymography. Medium conditioned by hMMP-3-transduced 293 cells, or by non-transduced, Ad.ßgal- or hMMP-3-transduced HASMC was used. A lysis band of 57 kDa consistent with the M_r of pro-MMP-3 was present only in Ad.MMP-3 transduced cells. Transduced HASMC grown on the amniotic membrane (transfected/a) showed higher MMP-3 caseinolytic activity than cells grown on plastic.

In situ-zymography. MMP-3 activity was analyzed by in situ casein zymography. Vessels were snap frozen in OCT medium after harvesting and cut into 8-μm sections. The sections were put onto microscope slides and incubated at 37°C for 48 hours in Tris 50 mM, CaCl2 10 mM, Brij 35 0.05% containing agarose 1% and resorufin-labeled casein (10 mg/mL; Boehringer, Mannheim, Germany). Casein degradation was observed with a fluorescence microscope.

Measurement of neointima formation. Neointima formation was quantified on cross sections of vein grafts (6/group) stained with elastica van Gieson (EvG) and Mayer's hematoxylin and eosin (H&E). For randomization, vessels were longitudinally divided into 6 regions, and from each region the first cross section was picked for analysis. Intima, media, internal, and external elastic lamina were identified and traced on digital images of sections cut at five spaced intervals using Image-Pro Plus (Version 4.1.5, Cybernetics Inc, Silver Spring, Md). The investigator was blinded since labeling of cross sections did not allow identification of experimental group. Three parameters were calculated: stenosis degree (intima-lumen/lumen), intima/media ratio (intima-lumen/media-lumen) and lesion thickness (radius of the media-radius of the intima).

Statistical analysis. Statistical analysis was performed by one-way analysis of variance (ANOVA) combined with post-hoc test LSD using SPSS (Version 12.0, Chicago, Ill). Any $P < .05$ values were considered statistically significant.

RESULTS

HASMC transduced with Ad.MMP-3 overexpress functional MMP-3. To study the role of MMP-3 in the generation of intimal hyperplasia, we used adenovirus mediated gene transfer to upregulate MMP-3 expression in aortic smooth muscle cells. By Northern blotting, hMMP-3-transduced HASMC had high levels of MMP-3 mRNA, whereas control, non-transduced, or Ad.ßgal-transduced cells showed no such transcript (Fig 1, *a*). Western blotting with MMP-3 antibody showed expression of a 57-kDa band consistent with the M_r of pro-MMP-3 (Fig 1, *b*). This band was undetectable in the CM of control cells, showing that non-transduced HASMC expressed no MMP-3. A similar amount of MMP-3 was present in the CM of HASMC grown on the amniotic membrane (Fig 1, *b*, SMC transduced/a). In vitro experiments with different adenoviral vector concentrations showed the highest transduction efficiency of 24.1% and the lowest cytotoxicity with 2×10^8 pfu/mL (100 pfu/cell). Dose response experiments showed no relevant increase of transduction efficiency when vector concentration was higher. The growth rate of virus-transduced cells was slightly but not significantly higher that of non-transduced cells (data not shown), and no difference in viability was observed between transduced and non-transduced HASMC. Consistent with the Western blotting result, casein zymography of medium conditioned by hMMP-3-transduced HASMC showed a lysis band of 57 kDa (Fig 1, *c*). This caseinolytic band was converted to 45 kDa following incubation of the conditioned medium with 1 mmol/L p-aminophenylmercuric acetate (APMA) at 37°C for 2 hours, indicating activation of pro-hMMP-3 by cleavage of the pro-domain (data not shown). Thus, transduction of HASMC with hMMP-3 resulted in overexpression of functional hMMP-3.

Overexpression of MMP-3 inhibits HASMC migration. To evaluate the effect of MMP-3 gene transfer on SMC invasion of the extracellular matrix, MMP-3 transduced HASMC were seeded onto the stromal aspect of the amniotic membrane and SMC-migration was measured. In the presence of 10 ng/mL of PDGF, non-transduced or Ad.ßgal-transduced HASMC migrated into the collagenous stroma to the same extent. Cells were found at varying depth in the stromal meshwork; some cells in close contact

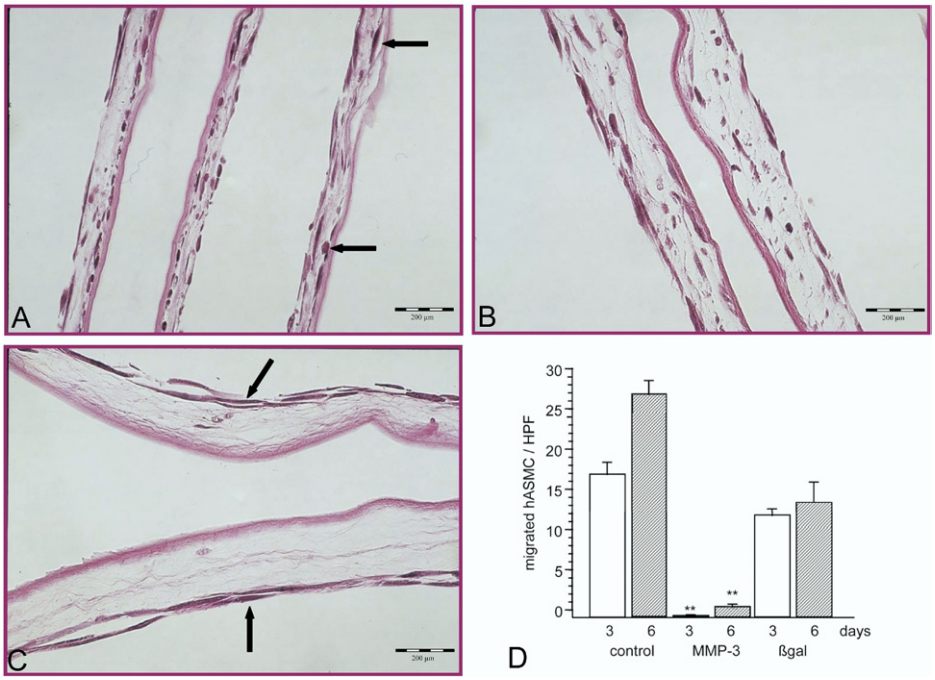

Fig 2. Migration of human aortic smooth muscle cells (HASMC) through the amniotic membrane mimicking the vascular extracellular matrix, stimulated with 10 ng/mL of PDGF. **a,** Untreated HASMC migrated from the surface into the stroma and were found in various depths of the matrix (*arrows*). **b,** HASMC were transduced with adenoviral vectors encoding ß-galactosidase (AVEßgal) before seeding. Cells migrated in comparable numbers into the stroma as untreated HASMC. **c,** When transduced with hMMP-3 before seeding, HASMC rarely invade the stroma and remain on the surface (*arrows*). **d,** Number of migrated HASMC was counted by 10 random microscopic high power fields (HPF) per histologic sections. Difference between MMP-3-overexpressing HASMC and controls was highly significant (** $P < .001$), but not between controls. Differences in thickness of the membrane (**c**) relate to natural variability of amnion membrane. Hematoxylin and eosin (H&E), magnification 400× for all photographs. Mean ± standard deviation is shown **(d)**.

with the stromal side of the basement membrane (Fig 2, *a, b*). In contrast, only occasional Ad.MMP-3-transduced HASMC were detected in the superficial layers of the stroma (Fig 2, *c*). Counting of the cells in 10 random high-power (400×) microscopic fields showed only single MMP-3-transduced HASMC in the stroma after 3 or 6 days of incubation (Fig 2, *d*/graph). In contrast, up to 20-fold more non-transduced or Ad.ßgal-transduced HASMC were counted under the same conditions ($P <$ .001). These results indicated that high levels of MMP-3 expression inhibit SMC invasion of a collagenous stroma.

Ex vivo transduction of autologous vein grafts with hMMP-3 inhibits the development of intimal hyperplasia in vivo. To investigate the role of MMP-3 in the development of intimal hyperplasia we transduced jugular vein explants ex vivo and grafted them into the carotid artery as described under Materials and Methods. Analysis of the venous grafts by immunocytochemistry and in situ zymography 7 days after implantation showed that adenovirus-mediated gene transfer of hMMP-3 resulted in sustained overexpression of active hMMP-3. By immunocytochemistry MMP-3-transduced grafts showed prominent staining in the intima and faint staining in the media (Fig 3, *a*). Virtually, the whole luminal lining of the vessel was stained, indicating high transduction efficiency. Conversely, non-transduced control grafts showed no specific staining. Similarly, in situ casein zymography showed high proteolytic activity localized to the intima of MMP-3-transduced grafts (Fig 4, *a*) and no caseinolytic activity in control grafts incubated with PBS alone or transduced with AVEßgal (Fig 4, *b, c*). Thus, ex vivo transduction of vein explants resulted in high and sustained levels of expression of active MMP-3.

To analyze the development of intimal hyperplasia, vein grafts were harvested 28 days after implantation, and cross sections were characterized histologically. Significant intimal hyperplasia was detected in control vein grafts incubated with PBS or Poloxamer 407 (Fig 5, *a, b*; Fig 6). Similarly, control vein grafts transduced with ß-gal showed development of intimal hyperplasia comparable to the other controls (Fig 5, *c*). Conversely, intimal hyperplasia was significantly reduced in vein grafts transduced with MMP-3 (Fig 5, *d*), as assessed by measurement of stenosis degree ($P = .001$), intima/media-ratio ($P = .023$) and lesion thickness ($P = .003$) (Fig 6). Therefore, these results showed that overexpression of MMP-3 inhibits the development of intimal hyperplasia in arterialized vein grafts.

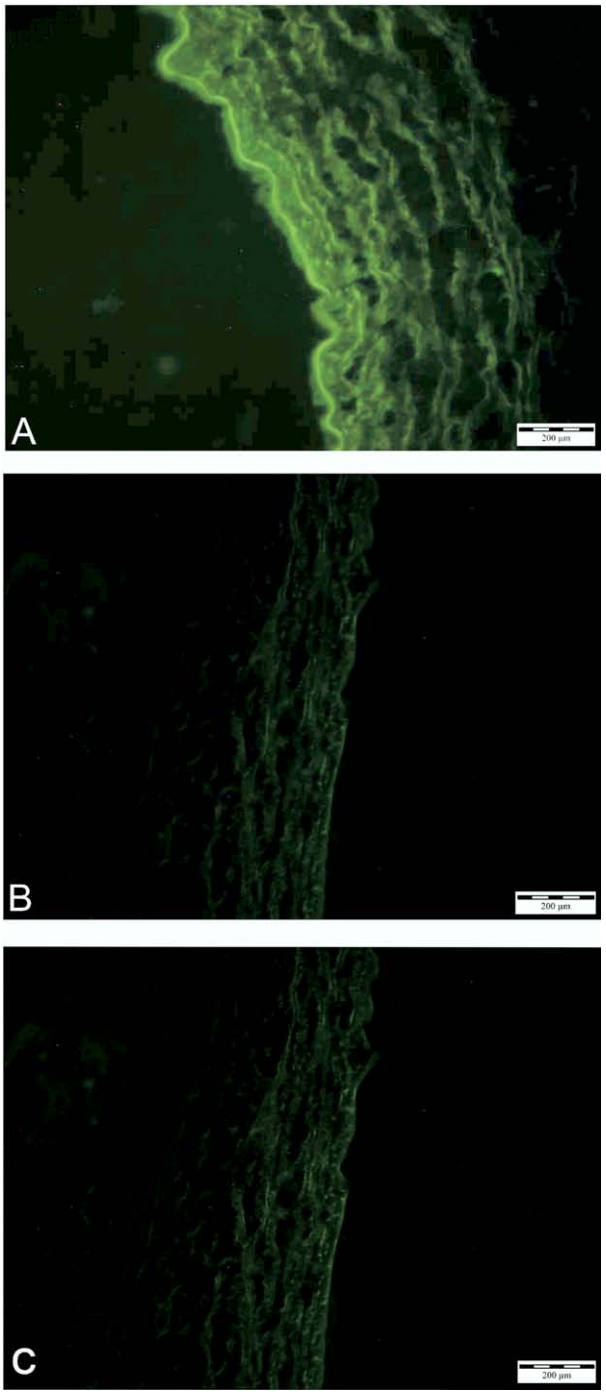

Fig 3. Immunocytochemical analysis of matrix metalloproteinase (MMP)-3 in cross-sections of vein grafts 7 days after implantation. **a,** Vein graft transduced with human matrix metalloproteinase (hMMP)-3. **b,** Non- transduced control. **c,** Vein graft transduced with hMMP-3 stained with secondary antibody alone.

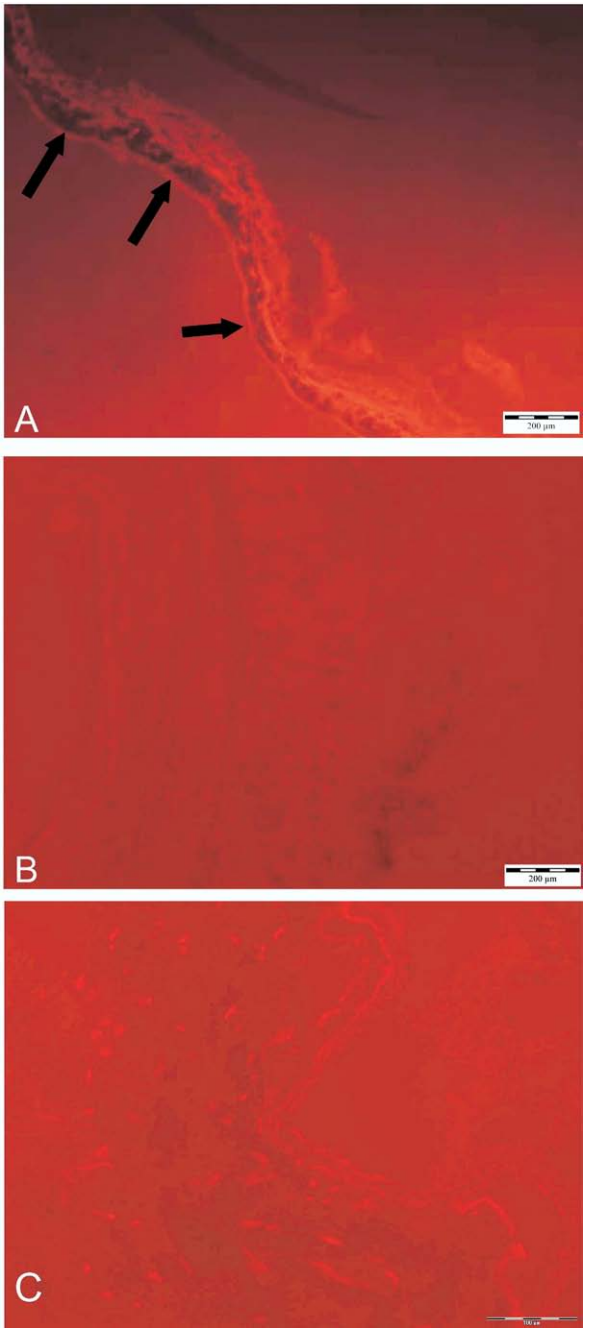

Fig 4. In situ casein zymography of vein grafts 7 days after implantation. **a,** Vein graft transduced with human matrix metalloproteinase (hMMP)-3. Dark areas in the intima (*arrows*) indicate digestion of red-labeled casein. **b**, Non-transduced control. **c**, Adenoviral vectors encoding ß-galactosidase (AVEßgal) transduced control. No caseinolytic activity is visible in the two controls.

DISCUSSION

The data presented show that adenovirus-mediated overexpression of stromelysin-1 (MMP-3) reduces migration of HASMC in an in vitro model of the vascular wall, and causes reduced development of intimal hyperplasia in vivo. These findings contrast with the understanding of the role of MMP-3 in the development of intimal hyperplasia.

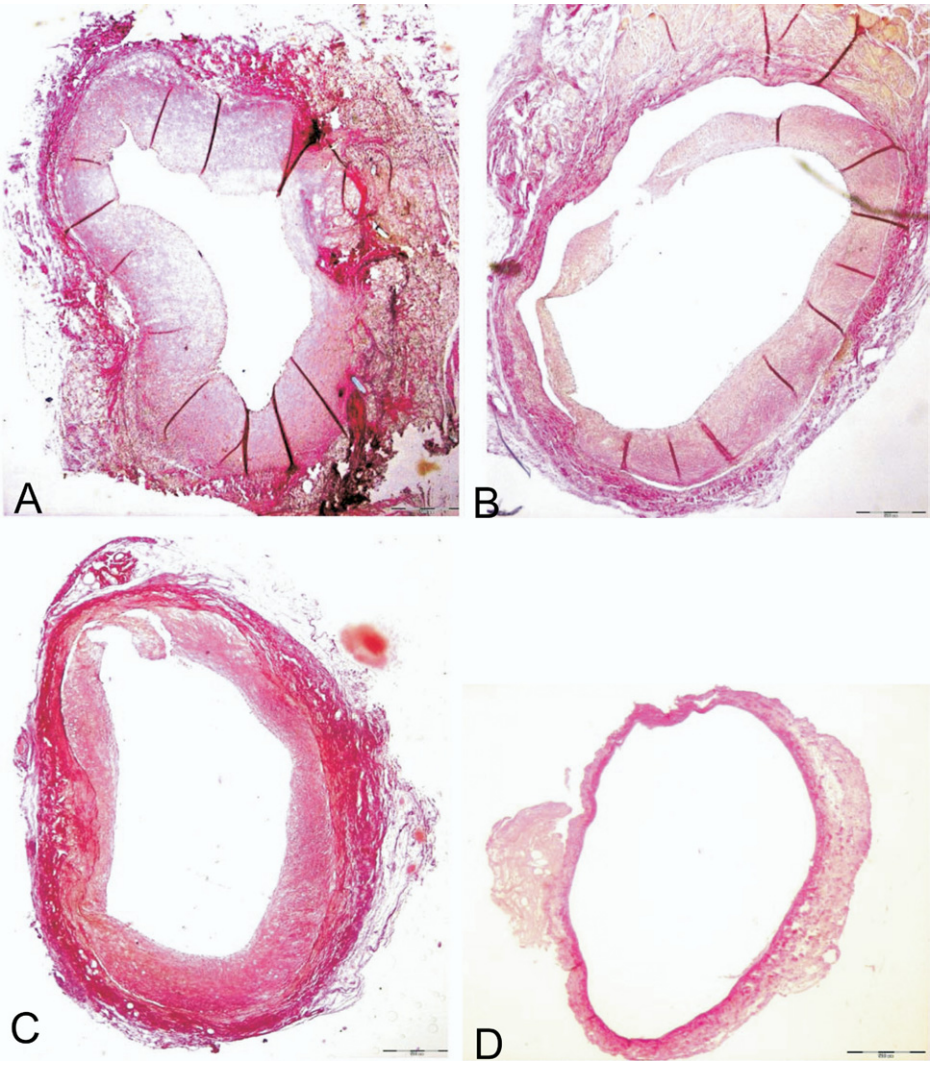

Fig 5. Development of intimal hyperplasia in vein grafts 28 after interposition into the carotid artery. Elastica van Gieson, magnification 20×. **a,** Graft incubated with PBS before implantation, showing severe neointima formation. Comparable amounts of intimal hyperplasia were found in vein grafts incubated with Poloxamer 407 **(b)** or transduced ex vivo with adenoviral vectors encoding ß-galactosidase (AVEßgal) **(c)** before implantation. Vein grafts transduced with human matrix metalloproteinase (hMMP)-3 showed almost no intimal hyperplasia 28 days after implantation **(d)**.

The broad substrate specificity and strong proteolytic activity against components of the extracellular matrix led to the assumption that – like other proteinases – MMP-3 promotes SMC migration and development of intimal hyperplasia. High levels of MMP-3 have been described in atherosclerotic plaques and in the SMC of atherosclerotic coronary arteries.[10,11] Mechanical injury of rabbit vascular smooth muscle cells (VSMCs) was associated with induction of stromelysin-1 mRNA expression, migration and proliferation, and neointima formation after vessel wall injury of rat carotid arteries was substantially inhibited by antisense oligonucleotides to stromelysin-1 mRNA.[21,22] All these findings supported the hypothesis that MMP-3 promotes SMC migration and development of intimal hyperplasia.

However, other studies have shown that reduced expression of MMP-3 in patients with a common 6A/6A polymorphism leads to increased progression of coronary atherosclerosis,[12] whereas the 5A/6A polymorphism with higher MMP-3 levels is associated with reduced restenosis after percutaneous transluminal coronary angioplasty (PTCA) and stenting compared to patients with 6A/6A alleles.[13] It has been speculated that MMP-3 has a dominant protective effect from lumen loss associated with the 5A allele and consecutive higher MMP-3 levels. Consistent with this hypothesis, healthy male subjects homozygous for the 6A allele (ie, with low MMP-3 expression) show increased wall thickness, enlarged arterial lumen, and local reduction of wall shear stress, which might predispose them to atherosclerotic plaque formation.[23] Studies with

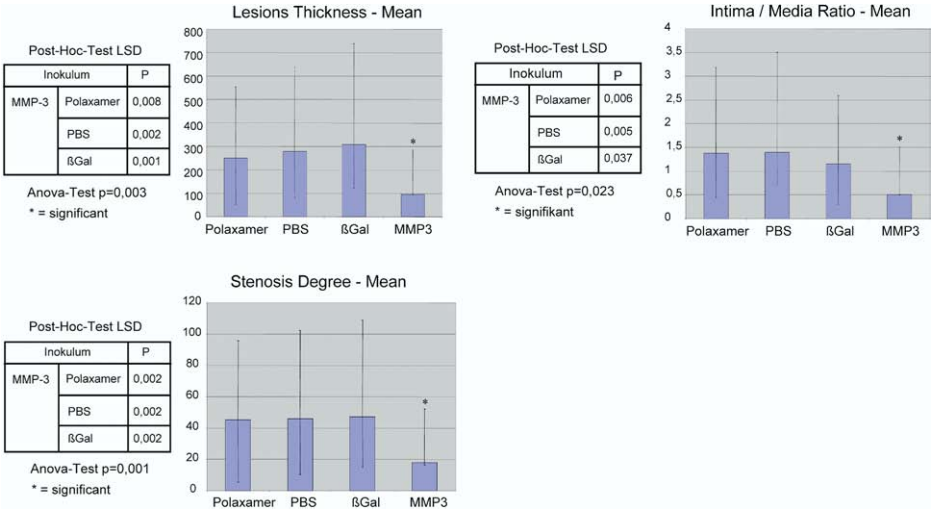

Fig 6. Degree of intimal hyperplasia in vein grafts 28 day after implantation. N = 6 animals per group were investigated. Lesion thickening, intima/media ratio and stenosis degree were measured as detailed under Materials and Methods. Mean ± standard deviation is shown.

apoE$^{-/-}$ MMP-3$^{-/-}$ double knock out mice also support the hypothesis of a protective function of MMP-3 in vascular remodeling. Atherosclerotic plaques in these animals are four times as large as in apoE$^{-/-}$: MMP-3$^{+/+}$ mice.[14,24] These findings support the hypothesis that high levels of MMP-3 have a protective role in vascular remodeling.

In our study, both migration of HASMC and development of intimal hyperplasia were reduced by overexpression of MMP-3. Initially, we observed reduced migration of HASMC overexpressing MMP-3 in a Boyden chamber assay (data not shown). Therefore, to study SMC migration in a more physiological setting we used a cell migration model based on the amniotic membrane.[16] The composition and structure of the amnion extracellular matrix is more similar to that of vessels than is Matrigel (BD Biosciences, San Jose, Calif). The amniotic membrane has a dense basement membrane and abundant collagenous stroma with elastin fibers, which confer great resistance to traction and resilience on the tissue, making it more similar to a vessel than Matrigel. Because of the harsh treatment used to remove the epithelial layer (incubation in 0.25 M NH4Cl for 1 hour), the occasional fibroblasts present in the amnion stroma are destroyed, leaving an acellular collagenous matrix. Therefore, in this model the cells that are found in the stroma are the viable cells that invaded from the stromal surface. We found that stimulation with PDGF induced SMC migration and invasion into the amnion stroma, a process blocked by MMP-3 overexpression. For practical reasons, we used arterial instead of venous SMC for our studies. Venous SMC tend to exhibit increased proliferation and migration compared to arterial SMC.[25-27] However, although we used less motile HASMC, migration was upregulated by PDGF both in untreated and Ad.ßgal-transduced cells but not in MMP-3-transduced cells.

The vein graft model of intimal hyperplasia that we used showed significant reduction of intimal hyperplasia in grafts transduced with hMMP-3 relative to non-transduced or Ad.ßGal-transduced controls. Immunocytochemical and in situ zymography analysis showed that MMP-3 overexpression in transduced grafts lasted at least 7 days. We did not characterize MMP-3 expression at 28 days after grafting, the time when intimal hyperplasia was analyzed. Since adenoviral expression is transient, overexpression is unlikely to last longer than 4 weeks after transduction. However, the early development of IH occurs in the first 2 weeks after venous graft arterialization;[28,29] whereas the late remodeling of the arterialized venous graft is essential for adaptation of the vessel wall to the high pressure system. Experience with drug eluting stents in percutaneous catheter interventions (PCI) for coronary artery disease shows that the benefit of early inhibition of intimal hyperplasia lasts longer than the drug eluting effect for prevention of stent stenosis. However, from our experiments we have no information if longer overexpression of MMP-3 is required for long-term reduction of intimal hyperplasia in arterilized vein grafts.

Gene transfer with adenoviral vectors is a potentially useful technique for this clinical application. For the treatment of vein grafts, the adenoviral vectors are used only ex vivo, and grafts are flushed several times after transduction for removal of free adenoviral vectors. Therefore, only trace amounts of viral vectors are expected to remain in the vein graft. As long as large numbers of infectious adenoviral vectors are not injected systemically, the risk for the patient is extremely low. There are no reports that topical ex vivo administration of adenoviral vectors results in systemic complications. Furthermore, to improve the safety for possible clinical application, we used a relatively low number of viral infectious units and a cell-system for vector production that makes the development of replication-competent ad-

enoviral (RCA) vectors almost impossible. However, a perfect delivery vehicle for gene transfer still remains unavailable.

The mechanism by which MMP-3 reduces SMC-migration and intimal hyperplasia remains unclear. The observation that relatively low transduction efficiency resulted in almost complete reduction of migration in vitro, presumably including uninfected cells, suggest that the underlying effect is not cell-specific. It is possible that MMP-3 degrades PDGF, thus depriving vascular smooth muscle cells of the major inducer of cell migration. However, this hypothesis has not been tested. Based on existing reports, we speculate that excess stromal degradation by high levels of MMP-3 deprive SMC of anchorage sites required for migration. This hypothesis is consistent with previous findings that inhibition of cell attachment to Matrigel inhibits cell migration.[30] Fibronectin (FN) mediates cell adhesion, migration, and cytodifferentiation,[31] and SMC uses FN to bind to type I and III collagens.[32] Intact FN (and laminin) is therefore essential for SMC migration. Because FN and laminin are substrates for MMP-3, it is possible that excess degradation of these (and other) extracellular matrix proteins by MMP-3 results in impaired SMC migration. The theory of "no grip, no invasion" has been discussed elsewhere[33] and is supported by findings that excessive proteolysis can cause damage to the tissue and dissolve the matrix needed for anchoring the migrating cells.[34,35] This view is also supported by a recent study showing that MMP-3 knockdown skeletal muscle satellite cells migrated faster than controls.[36]

MMP-3 can promote extracellular matrix degradation directly and indirectly by activating other MMPs, including MMP-9 and MMP-7.[8,18] The collective action of these MMPs in the graft may, therefore, cause excessive degradation of the extracellular matrix, generating microenvironmental conditions that are not permissive for SMC migration.

In addition, MMP-3 may play a role in the chemotactic recruitment of a number of inflammatory cell types directly[37,38] or through the chemotactic action of protein (eg, laminin) degradation products.[39] Additional secretion of MMPs (eg, MMP-9) from attracted macrophages will result in increased proteolytic activity.

Thus, a variety of observations support the concept that MMP-3 has beneficial effects on venous vascular remodeling in the setting of venous grafting. Further studies are warranted to clarify the role of MMP-3 in plaque rupture, restenosis, and narrowing of arterialized autologous vein grafts, and to investigate the therapeutic potential of MMP-3 overexpression in the vascular wall.

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AUTHOR CONTRIBUTIONS

Conception and design: KK, AHe, PM, AHa
Analysis and interpretation: KK, AHe, MK, PM
Data collection: KK, RS
Writing the article: KK, PM
Critical revision of the article: KK, AHe, MK, PM, AHa
Final approval of the article: KK, RS, AHe, MK, PM, AHa
Statistical analysis: KK, RS
Obtained funding: KK, PM, AHa
Overall responsibility: KK

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